U.S. WHEAT & BARLEY SCAB INITIATIVE VDUN GENOTYPING WORKSHOP
AGRICULTURE AND AGRI-FOOD CANADA, CEREAL RESEARCH CENTRE
WINNIPEG, MB CANADA
MAY 11, 2004

Host

Daryl Somers	Agriculture and Agri-Food Canada, Winnipeg

USWBSI Team Members

James Anderson	University of Minnesota, Workshop Coordinator
Guihua Bai	USDA-ARS Genotyping Center, Manhattan, KS
Gina Brown-Guedira	USDA-ARS, Manhattan, KS
Shiaoman Chao	USDA-ARS Genotyping Center, Fargo, ND
Karl Glover	South Dakota State University
Carl Griffey	Virginia Polytechnic Institute and State University
Herb Ohm	Purdue University
Kevin Smith	University of Minnesota
Clay Sneller	Ohio State University
Stephen Szalma	USDA-ARS, Raleigh, NC (representing David Marshall)

Agenda (May 11, 2004)

8:30 Welcome from Dr. Peter Burnett, CRC Site Director
8:35 Introductions and opening remarks (Somers and Anderson)
8:45 Wheat breeding/Marketing in Canada (Doug Brown)
9:15 Doubled Haploid technology for wheat breeding (Julian Thomas)
10:10 AAFC molecular map and genotyping technology (Somers)
10:40 Molecular breeding of FHB resistance (Somers) and discussions of morning session
11:45 Lunch at the University Club
1:15 Tour of Facilities and demonstrations
    Genotyping lab
    Doubled Haploid facility
3:00 Coffee and Round Table discussion
6:00 Dinner at "The Round Table"

Purpose of Workshop:

Four USDA-ARS Genotyping Centers are being established at Raleigh NC, Fargo ND, Manhattan KS and Pullman WA. Research conducted at the Genotyping Centers can speed the short-term objective of bringing FHB resistant varieties into commercial production, and enhance the longer-term objective of utilizing diverse sources of resistance to combat FHB.

This was the first of three workshops designed to produce protocols for 1) the utilization of the Genotyping Centers in marker assisted selection for cultivar development, 2) coordinated multi-state research programs in QTL discovery and validation, and 3) coordinated multi-state programs in backcrossing of new sources of FHB resistance into adapted genetic backgrounds. Dr. Daryl Somers' lab was chosen as the first site visit because of his experience in using DNA markers, high-throughput genotyping capacity, and large multi-breeding program effort underway in his lab to rapidly introgress FHB resistance genes into Canadian germplasm.

Summary of Workshop:

Julian Thomas gave us a tour of facilities used in doubled haploid production and Doug Brown provided an overview of wheat breeding and marketing in Canada. The estimated cost

for double haploid production was $20 CDN/line (mostly salary) and production was 20,000 DH lines per year with 3 full-time technicians with alternating schedules to provide activity 7 days per week.

Dr. Somers' lab works with DNA markers using modern genotyping equipment and robotics. He employs 7 technicians. Major equipment includes an ABI 3100 sequencer (16 capillary machine, used for SSR fragment sizing, can also be used for SNP genotyping and sequencing), six 384-well thermocyclers, modified paint shaker for DNA extraction, TECAN Freedom EVO robot (recently installed), and Rainin digital repeating pippetors. SSR markers are used almost exclusively with some effort in STS and SNP markers. They use four and five-color systems on the ABI (3 or 4 for samples plus 1 for size standard in each lane).

Samples in the lab are handled as part of projects – he does minimal service work on a "trade-off" basis. Currently, there is a nationwide effort in Canada to introgress multiple FHB resistance genes into multiple genetic backgrounds using backcrossing and extensive use of DNA markers to track the genes of interest as well as select for the recurrent parent background. Key points/lessons learned are given below and my editorial comments/opinions are given in italics

1) If one 384 and one 96 well plate is run in ABI 3100 for 24 h a day in every working day, it can generate about 400,000 data points per year. Daryl estimated that 2-2.5 full-time technicians could achieve this level of output for PCR analysis if DNA is ready.

Small projects greatly reduce efficiency and throughput. Since most cooperating breeders have access to equipment to run small samples, these should enter the genotyping labs only in rare circumstances. This will allow the genotyping labs to utilize their resources on larger projects (large-scale screens and mapping populations). We will need to discuss an appropriate cut-off for sample no. (96 may be a good cut-off because most samples will be handled in 96 well plate format for DNA extraction and possibly other downstream processing).

2) Breeder partners send either crushed half seeds (non-embryo half) or lyophilized leaves in 96-well format. This helps the genotyping lab in several ways:

- breeder is responsible for correctly loading the 96-well plate
- reduces labor and resources contributed by the genotyping lab
- assures "buy-in" from breeder on a project

For US Genotyping Centers, this should help control sample number because this takes time and requires active involvement by the breeder. Clear communication between the breeder and genotyping lab is essential. A standardized protocol (web-based) for submitting samples should be used.

3) Bottlenecks/processing time

- Cutting half-seeds takes about 2 hours/96-well tray, leaf tissue about 1 hour
- Repeating 8-channel pipettes are essential if robot is not available
- Doesn't do any multiplexing in PCR, just pooling of samples with different color tags (fluorescent labeled PCR products) for running on the ABI 3100.
- Data processing/archiving takes a lot of time – about 1/2 day for each full day of ABI 3100 operation
- Current bottleneck is the ABI 3100, the most expensive piece of equipment to buy and operate

The bottleneck at the genotyping labs is more likely to be adequate staffing.

4) Software for managing/archiving/reviewing data is critical.

The genotyping centers should decide on a similar platform and either modify an existing program or commission their own to suit their needs. Personnel at one of the four labs should take the lead on this for all the labs – the logical choice is S. Chao because of her recent bioinformatics and electronic sample tracking background.

5) Projects in the lab are completed according to their respective deadlines (not necessarily first in/first out)

This will be more difficult for the genotyping centers as they will be working with more breeders. Communication and software will have to be optimized to avoid problems with sample turn-around time. Also, because genotyping center regions are defined based on wheat market class and all barley is in the Fargo region, outsourcing to other genotyping labs during busy periods should be considered. Using common software and genotyping protocols will facilitate this. A natural periodicity between MAS breeding projects and genetic investigations should occur based on the biology of the crop(s) the genotyping centers are working with.

6) Estimated costs per data point for analyzing PCR products in ABI 3100 were $0.30-0.40 CDN for consumables and $0.40 for labor, assuming that 5 color multiplex PCRs are loaded and ABI 3100 runs daily. This converts into approximately $0.50-0.60 US.

7) He developed a consensus map of SSR markers that has been very helpful for finding markers in regions of interest. This work has been accepted in *Theoretical and Applied Genetics*.

8) Daryl Somers and Stephen Fox stressed the importance of having the "exact" parents for use in parental polymorphism screens

This concept is extremely important. Surveying the entire genomes of diverse set of small grains inbred representing each crop species will assist in marker-based mapping projects and MAS breeding efforts in the long term. This approach also is applicable for identification of favorable loci for quantitative traits.

9) Linkage drag is a concern. Most of their selected intervals are 15-40 cM, their goal is < 10 cM.

This can only be improved through increasing the number and density of available molecular markers.